The Safety of 17α-Methyltestosterone Medicated Feed to Tilapia

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Introduction

- The use of 17α-methyltestosterone (17MT) in tilapia farming dates from the late 1960's when various hormones and treatment methods were tried in order to produce single sex tilapia stocks to overcome the widespread problem that they reproduce excessively.

- Hormonal sex-reversal techniques were developed in the 1970s.

- Use of 17MT to induce sex reversal in tilapia is a common practice in many parts of the world. Legal?

- 17MT is used in US aquaculture under an INAD exemption.

- Gonadal differentiation occurs 8-25 days post-hatch, depending on environmental conditions.

- If females receive 17MT in their feed during this time, they will develop as phenotypic males.

- So, 17MT administered to newly hatched fry (3-12 days old) for 28 consecutive days results in male populations.

- Why? Male tilapia grow ~ twice as fast as females.

- Reproduction is eliminated so fish spend their energy growing.

Safety to consumers

- 17MT is a synthetically produced anabolic and androgenic steroid hormone; so it promotes both muscle growth and the development of male sexual characters.

- Anabolic hormones are widely used in agriculture to promote weight gain in cattle and sheep.

- Commercial hormone implants for livestock contain a mixture of both natural and exogenous male and female hormones.

- These implants contain relatively large quantities of hormone which are released continuously over several months.

- No withdrawal time for the implant is required before the animals are slaughtered. (?)

- Meat and milk can still contain detectable levels of hormones.

Safety to consumers (continued)

- Tilapia production involves exposing only young fry to < 0.02 mg 17MT per tilapia in total.

- 17MT is rapidly eliminated within days to undetectable levels in the viscera, carcass, blood and muscle.

- Sex reversed fish are reared until they reach marketable size, by which time no hormone residue remains.

- Nile tilapia *Oreochromis nilotica* is the main species farmed commercially in the world.

- We used Nile tilapia hybrid for this study.

- This study evaluated the safety of 17MT-medicated feed on tilapia at 0, 9, 27, and 45 mg MT/kg body weight/day for 28 consecutive days.

Materials & Methods

Experimental Design

- Four treatments; surrogate tanks were used to determine feed rates of this GLP study.
- Four replications per treatment.
- Twenty 18 L glass aquaria held 15 L of well water; recirculated with aquarium filters.
- Aquaria were cleaned daily and 20% of the water was replaced until the last week.
- Fry were fed Tilapia Starter Diet #0 at 15% body weight/day; four feedings daily.
- Medicated feed was prepared at Rangen Feeds (Buhl, ID) by top-coating.
- Feed samples from each treatment were collected before, during and after the experiment for 17MT dose verification.

Pre-Experiment

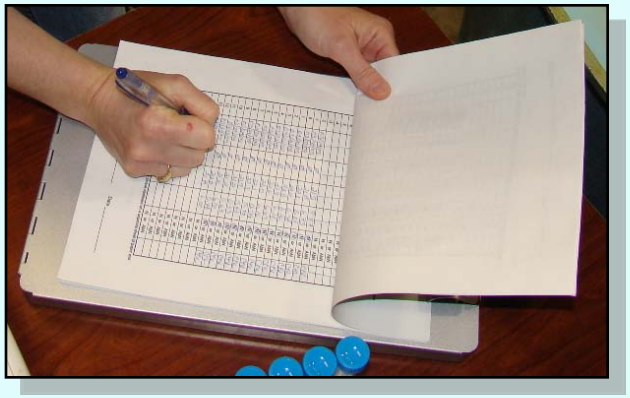
- Nile tilapia hybrid sac-fry were obtained from AmeriCulture, Inc. (Animas, NM) and acclimated for 3 days.
- Weight/length – 0.0123 g and 9.8 mm.
- Baseline fish health and histopathology was observed:
  - Gross necropsy (20 fish)
  - Histology samples (10 fish)
- Fish were randomly allocated (50 fish/tank) and acclimated for 5 days.

Experiment

- Personnel maintaining/feeding fish were unaware of treatment assignments.
- Tanks were cleaned prior to feeding; temperature and DO were measured (am).
- Fish were fed medicated diets four times daily for 28 days.
- Fish in surrogate tanks were weighed weekly; feed amounts were adjusted accordingly.

Experiment

- Appropriate amounts of feed were measured into centrifuge tubes and refrigerated.
- General behavior/feed consumption were documented after two feedings/day.
- Fish that died were preserved (if possible) for histology.
- Ammonia and nitrites were measured twice weekly in each test tank.
- Alkalinity, hardness and pH were measured weekly.



Post-Experiment

- Tanks were processed sequentially.
- All fish from an individual tank were:
  - Weighed
  - Measured
  - Necropsied
- Necropsy included observations of body surface, gills and eyes.

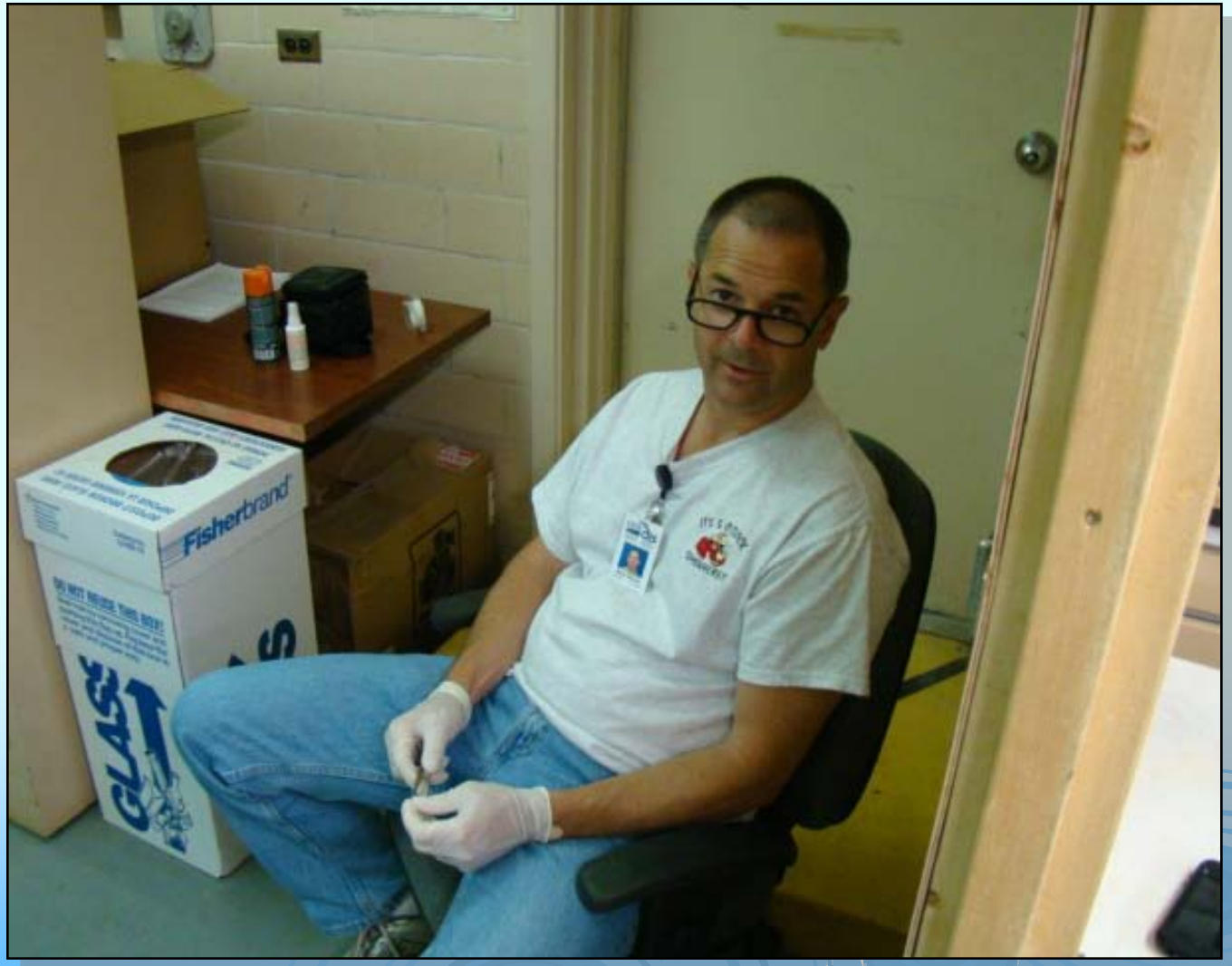
Post-Experiment (continued)

- Twenty fish were randomly selected and fixed in Davidson's solution.
- All tanks were processed in the same manner.
- Surrogate fish were euthanized.
- Fixed samples were shipped to AADAP for processing and histological evaluation.

Post-Experiment (continued)

- Tissues checked during histology will include gill, liver, kidney, skin, muscle, brain and heart.
- Tissues will be evaluated for lesions or cellular changes from 17MT-induced toxicity.
- Ten fish from each tank will be examined.
- Tissues will be scored as: no change, normal, mild, moderate, marked or severe.

Results & Discussion



Results & Discussion (continued)

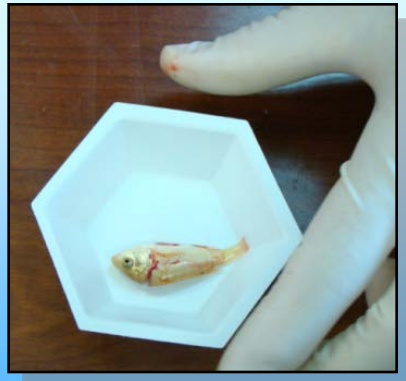
- Initial dose verifications were 60 mg/kg = 52 mg/kg, 180 mg/kg = 162 mg/kg, 300 mg/kg = 282 mg/kg.

- Weight and length – 2.11 g and 49.7 mm (initially 0.0123 g and 9.8 mm).

- Water Quality during experiment:
  DO = 6.7 mg/L, temp = 28°C, alkalinity = 231 ppm, hardness = 131 ppm, pH = 8.3, TAN = <4.8 ppm, NO_2-N = <3.3 ppm.

- Necropsy and histology in the reference population will be considered “normal” for tilapia fry reared under experimental conditions.

- Sequence of histology observations will be: pre-study, 0 mg/kg, 45 mg/kg, 27 mg/kg.



Results & Discussion (continued)

Control treatment

- Fish health - normal tissues
- Survival – 96.5 4.1%

9 mg/kg treatment

- Fish health - normal tissues
- Survival – 91.0 7.4%

27 mg/kg treatment

- Fish health - normal tissues
- Survival – 97.5 1.9%

45 mg/kg treatment

- Fish health - normal tissues
- Survival – 80.0 ± 19.9% (94,100, 66 and 60%)

Results & Discussion (continued)

Cause of Mortality?

Cannibalism?

Conclusions

The Safety of 17α-Methyltestosterone to tilapia is at least 27 mg 17MT/kg body weight/day when administered for 28 consecutive days.

Reasons:

TBD

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